

REVIEWS

CITICOLINE SUPPLEMENTATION IN ISCHAEMIC STROKE: MOLECULAR BIOMARKERS FOR EVALUATION OF THERAPEUTIC RESPONSE

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ABSTRACT

INTRODUCTION: Ischaemic stroke (IS) is a complex neurological disorder involving multifactorial molecular and cellular responses that lead to neuronal damage and functional deficits. Despite progress in diagnostics and treatment, supplementary neuroprotective approaches and sufficiently informative biomarkers to assess therapeutic outcomes remain a challenge for scientists and clinicians. Citicoline (cytidine-5'-diphosphocholine) has gained attention as a multiple-action agent with membrane-stabilising, anti-inflammatory, antioxidant, and neuroregenerative effects.

AIM: This review aims to provide a comprehensive integration of available data on citicoline supplementation in IS patients, as well as molecular biomarkers associated with therapeutic response.

METHODS: A literature search was conducted in PubMed, ScienceDirect, and ResearchGate using pre-defined keywords related to citicoline, ischaemic stroke, neuroprotection, and molecular biomarkers.

RESULTS: The review summarises evidence regarding biomarkers associated with citicoline responsiveness, including SIRT1, BDNF, glutathione (GSH), malondialdehyde (MDA), cardiolipin, arachidonic acid, 8-isoprostaglandin F_{2α}, and trimethylamine-N-oxide (TMAO), and their relevance to monitoring therapeutic outcomes

CONCLUSION: The integration of citicoline-therapy-sensitive biomarkers can be considered reliable within the framework of precision medicine.

Keywords: citicoline, ischaemic stroke, biomarkers, neuroprotection, oxidative stress

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INTRODUCTION

Ischaemic stroke (IS) remains a leading cause of mortality and long-term disability worldwide, driven by a complex pathophysiological cascade involving neuronal, glial, vascular, and immune components (1,2). The process initiated by cerebral ischaemia is characterised by a rapid depletion of oxygen and glucose, leading to energy failure, ionic imbalance, and excitotoxic neuronal injury. This cascade

progresses through oxidative stress, inflammation, blood–brain barrier (BBB) disruption, and apoptosis (3). The therapeutic approaches for IS primarily aim to restore blood flow and neuronal function (4). In this context, neuroprotective agents capable of modulating the ischaemic cascade have gained considerable attention.

Cytidine 5'-diphosphocholine (CDP-choline) or citicoline is an essential endogenous intermediate in the phospholipid biosynthesis pathway, particularly in the production of phosphatidylcholine, a vital structural component of cell membranes, including neurons. For the first time, CDP-choline was obtained experimentally through crystallisation from yeast (5). In the 1970s, when the idea of its use as a pharmaceutical substance emerged, the name citicoline became widely adopted in scientific literature. The first scientific evidence of the neuroprotective effects of citicoline, administered as a pharmaceutical, was reported by Manaka et al. (1974), and since then, hundreds of studies have been published, based on clinical research and various experimental models (6). After being absorbed into the body, citicoline releases its two main components, cytidine and choline, which are distributed throughout the body, including crossing the BBB to reach the central nervous system (7). It is believed that its role in stimulating the synthesis of brain phospholipids underpins many of its beneficial effects (8). Additionally, it has been found that, when administered orally, citicoline is absorbed almost completely, with approximately the same bioavailability as when administered parenterally, and is well tolerated (8).

In addition of above mentioned, the identification of molecular biomarkers has become a key area of research aimed at improving diagnosis, prognosis, and therapeutic monitoring. Biomarkers provide measurable indicators of biological processes and therapeutic responses, and their incorporation into clinical practice may enable personalised treatment strategies (9).

This review summarises current knowledge on citicoline supplementation in IS, focusing on molecular biomarkers that indicate therapeutic response and the underlying biological mechanisms.

METHODS

A literature search was conducted on PubMed, ScienceDirect, and ResearchGate, covering publications up to March 2026. The following keywords were used in various combinations: “citicoline”, “history”, “ischaemic stroke”, “therapeutic potential”, “mechanism of action”, “molecular biomarkers”, “neuroprotective potential”, “supplement therapy”. Original research articles, clinical and experimental studies, systematic reviews, and meta-analyses focusing on citicoline, IS, and molecular biomarkers were considered eligible. Publications not directly related to IS, citicoline supplementation, or biomarker assessment were excluded. Priority was given to peer-reviewed studies providing mechanistic, clinical, or translational evidence relevant to therapeutic response monitoring.

RESULTS AND DISCUSSION

Molecular Mechanisms of Citicoline Action

Citicoline exerts its effects through several interconnected molecular mechanisms that directly target key components of the ischaemic cascade.

Lipid Metabolism

Citicoline is a precursor in the biosynthesis of phosphatidylcholine, a major component of neuronal membranes. During ischaemia, activation of cytosolic Ca^{2+} -dependent phospholipase A2 (cPLA2) leads to degradation of membrane phospholipids and the release of free fatty acids (FFA) into the serum and cerebrospinal fluid, including arachidonic acid (AA), which is metabolised into products that contribute to increased oxidative damage (10,11,12).

In this context, administration of citicoline enhances phosphatidylcholine synthesis and inhibits phospholipid degradation, thereby stabilising neuronal membranes (13). It also stabilises mitochondrial membranes by preventing the loss of cardiolipin, a key phospholipid essential for mitochondrial function (14). These effects not only preserve structural integrity but also reduce the availability of substrates for lipid peroxidation and inflammation.

Oxidative Stress and Antioxidant Effect of Citicoline

Oxidative stress plays a major role in the pathogenesis of stroke, and in the neurodegeneration it induces (15). Reperfusion after ischaemia is a crucial

factor in exacerbating oxidative damage at both the cellular and tissue levels.

Citicoline exerts its neuroprotective effects by mitigating oxidative stress through multiple mechanisms. One possible mechanism underlying the neuroprotective effect of citicoline is its positive influence on reduced glutathione (GSH) levels (16,17). Citicoline, similar to plant-derived polyphenols, has been found to maintain high levels of GSH, on the one hand by protecting it from oxidation and, on the other, by stimulating its synthesis (18,19). Reduced glutathione is one of the most important endogenous antioxidants, involved in the neutralisation of free radicals and peroxides, both directly via a non-enzymatic mechanism and as a cofactor of the enzyme glutathione peroxidase (GPx) (20). It has been suggested that oxidising choline to betaine might increase the levels of reduced cellular glutathione (18). Choline released from citicoline can be metabolised into betaine, which serves as a methyl donor in the conversion of homocysteine to methionine. Further, methionine is metabolised to S-adenosyl-L-methionine, which contributes to the sulfur pool required for the synthesis of glutathione (18).

Anti-Inflammatory and Neuroprotective Mechanisms

Inflammation is a key component of the pathogenesis of IS. The inflammatory response is a complex cascade of molecular events and interactions among pro- and anti-inflammatory mediators, including vasoactive peptides and amines, pro-inflammatory cytokines, eicosanoids, and acute-phase proteins, aimed at preventing tissue damage and restoring function. Ischaemic brain injury stimulates local production of tumour necrosis factor alpha (TNF- α) and interleukin beta (IL-1 β) by microglial cells (9,21). Elevated levels of these pro-inflammatory cytokines are associated with BBB damage, reactive oxygen species (ROS) production, and upregulation of adhesion molecules. Furthermore, inflammatory signalling in stroke stimulates the expression of inducible isoforms of the pro-inflammatory enzymes cyclooxygenase (COX-2) and nitric oxide synthase (iNOS). COX-2 produces toxic prostanoids and superoxide radicals, which exacerbate post-ischaemic inflammation (22).

Neuroinflammation is a central driver of secondary injury in IS. Citicoline exerts anti-inflammatory effects through multiple mechanisms, including inhibition of nuclear factor kappa B (NF- κ B), a key transcription factor that regulates inflammatory gene expression (19). Through this mechanism, citicoline reduces the production of pro-inflammatory cytokines such as TNF- α , IL-1 β , and interleukin 6 (IL-6), while modulating microglial activation and limiting leukocyte infiltration across the BBB. Recently, increased expression of the NAD⁺-dependent deacetylase sirtuin1 (SIRT1) has been observed following citicoline treatment (23). The anti-inflammatory effect of SIRT1 is mediated by deacetylating NF- κ B (24). Increased *SIRT1* expression has been associated with ischaemic tolerance, helping to prevent stroke recurrence (25).

Biomarkers for Assessing Therapeutic Response to Citicoline

The molecular pathogenesis of IS involves a complex interplay of excitotoxicity, oxidative stress, inflammation, and apoptotic signalling. Therefore, identifying biomarkers to assess stroke severity and monitor successful therapeutic outcomes remains a major challenge. In addition, a high percentage of IS patients have severe comorbidities, such as diabetes, hypertension, and cardiac rhythm problems. Such patients undergo various therapies, making it significantly more difficult to assess the response to a specific one. Table 1 summarises data on some biomarkers whose levels may be indicative of IS, as well as data on the effect of citicoline therapy on these biomarkers.

The degree of oxidative stress can be assessed using biomarkers that are both products of free radical oxidation and antioxidants. Markers of oxidative damage to membranes include malondialdehyde (MDA), the end product of membrane lipid peroxidation, and isoprostanes, such as 8-iso-prostaglandin F₂-alpha (8-iPGF_{2 α}) (26). Isoprostanes are a family of prostaglandin-like molecules formed non-enzymatically via ROS-mediated peroxidation of AA, independently of cyclooxygenase activity. Clinical studies have demonstrated increased circulating levels of 8-iPGF_{2 α} in the plasma of IS patients (23). Levels of 8-iPGF_{2 α} are considered the gold standard for measuring cumulative oxidative damage provoked

by various factors, including neuronal stress (27). Formed endogenously, 8-iPGF_{2α} can be measured in various biological samples, including urine, blood plasma/serum, exhaled breath condensate, amniotic fluid, saliva, and tissue material (28,29).

Recently, a formulation combining citicoline with antioxidants showed significant potential to reduce MDA, 8-iso-PGF_{2α}, and AA in healthy controls (Table 1). The same study reported that the effect of supplementation on serum AA levels can vary by comorbidity (e.g., diabetes) but did not reduce 8-iso-PGF_{2α} levels in IS patients (23).

However, citicoline generally helps restore cellular and mitochondrial membranes. Administration of 500 mg/kg citicoline in experimental stroke significantly restored phosphatidylcholine (PC) and cardiolipin (CL) levels (Table 1) in experimental animals (18). Cardiolipin is a phospholipid specific to the inner mitochondrial membrane and plays an important role in maintaining neuronal homeostasis. Due to its unsaturated fatty acid content, CL can undergo lipid peroxidation under oxidative stress. Alterations in CL structure or a decrease in its total levels can lead to mitochondrial dysfunction (30). It has been reported that citicoline may stabilise mitochondrial integrity by inhibiting mitochondrial PLA2 activity and preventing cardiolipin loss (31,32).

One mechanism underlying the neuroprotective effects of citicoline is the activation of *SIRT1* gene expression (Table 1). The activity of the enzyme is associated with the regulation of many cellular functions, including maintaining metabolic homeostasis, extending lifespan, and reducing the risk of neurodegenerative diseases (33). Citicoline treatment has been shown to increase *SIRT1* levels in cultured neurons, circulating blood mononuclear cells, and in the brain (34). A similar positive effect on *SIRT1* expression has been demonstrated for plant polyphenols, which is considered one mechanism by which they help protect cells from oxidative stress and inflammation. Recently, a six-month intervention study using a supplement combining citicoline with aronia and green tea extracts reported that *SIRT1* expression was significantly upregulated in IS patients, with the most notable increase observed in those with diabetes comorbidity. A possible synergistic effect of the ingredients was suggested (23).

In addition to the above, citicoline was reported to regulate neurotrophic factors, a group of secreted proteins that play roles in the development, growth, survival, and differentiation of the central nervous system, as well as in maintaining its homeostasis (35). Brain-derived neurotrophic factor (BDNF) is the most abundant of these factors and is widely distributed throughout the nervous system (36). Furthermore, BDNF is considered an early indicator of nerve regeneration (37). Deficiency of BDNF is associated with severe pathophysiological symptoms in IS patients, underscoring its central role in ischaemia (38). BDNF-enriched exosomes derived from neural stem cells demonstrate therapeutic potential for the treatment of IS by effectively attenuating cerebral ischaemia-induced injury (39). Citicoline treatment significantly increases BDNF expression, thereby enhancing neuronal survival and regeneration as an innate defence mechanism in response to neuronal injury (Table 1).

Additionally, GSH plasma levels are generally decreased in IS, serving as a marker of increased oxidative stress and stroke severity (40). Citicoline treatment resulted in a significant increase in total glutathione levels (Table 1), an increase in glutathione reductase activity, and a decrease in the glutathione oxidation ratio following transient cerebral ischaemia in adult rodents (18). Citicoline treatment restores the glutathione redox ratio diminished in staurosporine-treated human neuroblastoma cells (41).

Malondialdehyde is a marker of oxidative stress and lipid peroxidation in the brain (Table 1). Redox status is typically assessed by measuring serum MDA levels. Elevated serum MDA levels indicate increased lipid peroxidation and inflammation and serve as a reliable, non-invasive biomarker (42,43). The capacity of citicoline to reduce MDA concentrations has been documented in both clinical intervention studies and experimental models of neurodegenerative disorders and brain injury (17). Inhibiting membrane lipid peroxidation is particularly important for preserving neuronal integrity under pronounced oxidative stress, such as that seen in cerebral ischaemia (44). In an intervention study, MDA levels were significantly reduced after six months of citicoline supplementation in IS patients and controls, with participants stratified by sex and lifestyle characteristics (45).

Table 1. Biomarkers indicative of IS and of response to citicoline therapy.

Biomarker	Biological Role	Changes in IS (Ref.)	Effects of Citicoline Supplementation (Ref.)	Type of the Study
AA	Polyunsaturated fatty acid, released from membrane phospholipids; precursor of eicosanoids and isoprostanes	Elevated (10,23)	Decreased (23)	Astrocyte cell culture (10) Human intervention study (23)
8-iPGF _{2α}	A marker of oxidative stress and lipid peroxidation, derived from AA	Significantly increased (23)	Decrease in healthy controls (23); Varies depending on the patients comorbidity (23);	Human intervention study (23)
CL	Major phospholipid in the inner mitochondrial membrane	Decreased due to reperfusion injury (18,47)	Restored levels due to PLA ₂ inhibition (18,32)	Experimental animal model (18) Human intervention study (23) Clinical trials (47)
PC	Major structural phospholipid in cell membranes, essential for membrane integrity and signalling.	Decreased due to PLA ₂ activation (18,48)	Donates substrates for PC synthesis, restores membrane integrity (13,18)	Experimental animal model (18) Human clinical study (13,48)
GSH	Cellular antioxidant neutralising hydrogen peroxide and lipid peroxides.	Decreased plasma levels (40,49)	Increased total GSH levels, increased glutathione reductase activity, decrease of glutathione oxidation ratio (18)	Experimental animal model (18) Human clinical study (40,49)
MDA	A marker of oxidative stress and lipid peroxidation in the brain	Significantly elevated levels (42)	Significantly decreased (45)	Human clinical study (42) Human intervention study (45)
SIRT1	Regulation of genes related to neuroinflammation and oxidative stress	Controversial: Decreased (50); Increased (51)	Increased markedly in IS patients with diabetes comorbidity (23)	Human intervention study (23) Human clinical study (50) Cell culture study (51)
BDNF	Role in the development, growth, cell survival, and differentiation of the central nervous system	Decreased levels (35,52)	Increases <i>BDNF</i> expression (37)	Experimental animal model (37) Human clinical study (35,52)
TMAO	Metabolite derived from gut microbiota metabolism of choline; linked to vascular and inflammatory processes	Significantly increased (46)	Indirect effect due to altered choline metabolism (53)	Cell culture studies (53) Experimental animal models (46,53) Human clinical studies (46,53) Human intervention studies (46)

Emerging evidence highlights trimethylamine-N-oxide (TMAO) as a potentially important biomarker linking gut microbiota metabolism with IS pathophysiology (Table 1). TMAO is generated through hepatic oxidation of trimethylamine, a metabolite produced by intestinal microbiota from dietary choline, phosphatidylcholine, and L-carnitine. Elevated circulating TMAO levels have been associated with endothelial dysfunction, platelet hyperactivity, vascular inflammation, and atherosclerotic progression, all of which contribute to increased IS risk. Clinical studies and meta-analyses demonstrate that patients with acute IS exhibit significantly higher plasma TMAO concentrations compared to controls (44,46). Since citicoline is a source of choline, concerns have emerged about its potential influence on TMAO production (7). Current evidence remains limited and partially indirect. Experimental and metabolomic studies suggest that choline-containing compounds may increase substrate availability for gut microbial trimethylamine production. However, available data indicate that citicoline administration does not necessarily result in clinically significant elevations of TMAO, possibly due to differences in intestinal metabolism, hepatic conversion efficiency, and concurrent modulation of oxidative and inflammatory pathways. Recent analyses suggest that citicoline-associated neuroprotective benefits may outweigh potential metabolic concerns, although further controlled clinical studies evaluating longitudinal TMAO dynamics during citicoline supplementation in IS patients are still required (7,46).

In summary, the observed variability in clinical outcomes may reflect differences in biomarker profiles among patients. For example, individuals with higher baseline inflammatory or oxidative stress markers may derive greater benefit from citicoline therapy. Importantly, the absence of universally effective neuroprotective agents in stroke highlights the need for biomarker-guided patient selection. Citicoline multimodal mechanism of action makes it a suitable candidate for such personalised approaches.

Limitations and Future Directions

Despite substantial advances in biomarker research in IS, several important limitations continue to restrict the translation of these findings into routine clinical practice. One major challenge is the con-

siderable heterogeneity of IS aetiology, clinical presentation, comorbidities, and treatment regimens, all of which may significantly influence biomarker expression and therapeutic response. In addition, many biomarkers demonstrate substantial temporal variability, requiring precise timing of sample collection and repeated measurements for reliable interpretation. Another critical limitation is the lack of standardised laboratory assays and validated reference ranges across different clinical centres. Certain highly promising biomarkers, including cardiolipin and specific lipid peroxidation metabolites, currently require sophisticated analytical platforms such as liquid chromatography-mass spectrometry (LC-MS/MS), which remain expensive, technically demanding, and not widely available in standard hospital laboratories (30,54,55,56). These methodological limitations considerably hinder their implementation in routine stroke diagnostics and therapeutic monitoring. Furthermore, many currently available studies are limited by relatively small patient cohorts, short follow-up periods, and insufficient integration of molecular biomarkers with neuroimaging and clinical outcome data.

Future research should therefore focus on large-scale multicentre prospective studies designed to validate biomarker panels with high diagnostic and prognostic accuracy. Integrating molecular biomarkers with imaging findings, genomic profiling, and artificial intelligence-based analytical approaches may substantially improve personalised therapeutic decision-making in IS. In addition, the development of cost-effective, standardised, and clinically accessible assays for advanced biomarkers such as cardiolipin, isoprostanes, and transcriptomic signatures could significantly facilitate the incorporation of precision medicine approaches into everyday neurological practice.

CONCLUSION

The integration of biomarker profiling with citicoline therapy is a promising step towards precision medicine in IS. However, further validation and standardisation are needed to translate these advances into routine clinical practice. Future research should prioritise large-scale, multicentre studies that integrate clinical, imaging, and molecular data. In addition, applying artificial intelligence and ma-

chine learning to biomarker analysis may help identify clinically relevant patterns and improve therapeutic decision-making.

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